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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:	)	
	)	
Xavier ROUAU, *et al.*	)	Group: 1651
	)	
Serial No.: 09/462,911	)	M. Meller
	)	
Filed: January 18, 2000	)	

For: A COMPOSITION COMPRISING AN ENZYME HAVING GALACTOSE OXIDASE ACTIVITY AND USE THEREOF

DECLARATION UNDER 37 C.F.R. § 1.131

Commisioner for Patents
Washington, DC 20231

Sir:

We Xavier ROUAU, Mette SCHRØDER, and Jørn Borch SØE, declare as follows:

1. We are the inventors named in the above-identified patent application.

2. We state that, prior to 1997, we reduced to practice in France, a WTO country, the invention of claim 33, attached hereto as Appendix A. This is shown by a copy (enclosed in Appendix B) of the following document, in which a time indication has been redacted:

Research Report authored by inventor Mette SCHRØDER and entitled "Production of substrate for galactose oxidase by depolymerization of arabinogalactan from wheat flour. 2nd Report from Study of the effect of enzymatic oxidizing systems (and of their combination with non-starch polysaccharide degrading enzymes) on flour components, dough and bread."

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3. The composition of claim 33 contains as a first component, a galactose oxidase (EC 1.1.3.9) and, as a second component one of the following:

(i) an oxidizable substrate for the galactose oxidase which is at least one of a galactan, a galactose oligomer or a galactose dimer,

(ii) an oxidizable substrate for the galactose oxidase including at least one of a galactan, a galactose oligomer or a galactose dimer, and an enzyme which is capable of converting a compound into a substrate for the galactose oxidase, or

(iii) an enzyme which is capable of converting a compound into a substrate for the galactose oxidase.

4. In section 3.8 of Annex B an experiment is described where galactan is subjected to oxidation by galactose oxidase. Thus, this description discloses that the invention of claim 33 (i) was reduced to practice prior to 1997.

5. In section 3.9 of Annex B a further experiment is described where a mixture of galactose oxidase, arabinogalactan and arabinofuranosidase is incubated under conditions where the arabinofuranosidase converts arabinogalactan into a substrate for the galactose oxidase as shown by the ABTS assay. Accordingly, this description discloses that the invention of claim 33 (ii) had been reduced to practice prior to 1997, i.e. a composition comprising galactose oxidase and an oxidizable substrate for the galactose oxidase including at least one of a galactan, a galactose oligomer and a galactose dimer, and an enzyme which is capable of converting a compound into a substrate for the galactose oxidase.

6. Additionally, the description of section 3.9. discloses reduction to practice of the invention of claim 33 (iii) prior to 1997, as initially the reaction mixture contained galactose oxidase, a compound (ararbinogalactan) that could be converted into a substrate for the galactose oxidase (galactan) and an enzyme capable of converting the compound into an oxidizable substrate for the galactose oxidase (arabinofuranosidase).

7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and

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belief are believed to be true; and further that these statements were made with the knowledge that willful statements and the like so made are punishable by fine and imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date 24 January 2002

ROUAU Xavier

Date

SCHRØDER Mette

Date

SØE Jørn Borch

Production of substrate for galactose oxidase by depolymerization of arabinogalactan from wheat flour

2. Report from:

Study on the effect of enzymatic oxidizing systems (and of their combination with non-starch polysaccharide degrading enzymes) on flour components, dough and bread

The presented work was carried out at Unité de Technologie des Céréales, INRA, Montpellier, under the supervision of Xavier Rouau.

Melte Schroder

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1. Introduction

In recent years a number of enzymes have been used as dough and/or bread improving agents. Examples of such enzymes are found within the groups of amylases, proteases, cellulases and pentosanases.
The high potential of certain pentosanases for bread improvement is limited by the detrimental effect on dough (stickiness, slackness) that appears for relatively low dosages of enzyme. This effect has been attributed to the occurence of excess of unbound water and extensive depolymerisation of pentosans (Rouau et al., 1994).
Addition of certain oxidizing enzymes can diminish the detrimental effect on dough, which means that higher levels of pentosanases can be used. The positive influence of the oxidation is thought to be related to cross-linking of pentosans through their ferulic acids and the disulfide bond formation in the protein fraction, although the respective importance of both mechanisms and the possible pentosan-protein network formation have not been studied in details.

The use of glucose oxidase as a dough improving additive has the limitation that this enzyme requires sufficient amount of glucose in order to be effective in a dough system and generally, the amount of available glucose in dough is low.
As an alternative to glucose oxidase as a dough improving additive might galactose oxidase be applied. The natural substrate for galactose oxidase in flours is the hydrolysis products of arabinogalactan. A compound of which no technological applications have been described so far.

In the present study, the depolymerisation of arabinogalactan is studied in order to produce oxidizable substrate for galactose oxidase in the dough.
The study is a part of a collaborative project between INRA (UTC) and Danisco Ingredients in which the effects of enzymatic oxidizing systems on wheat flour components, dough and bread are investigated.

1.1 Wheat arabinogalactan

Arabinogalactan-proteins are glycoproteins comprising of a galactose and arabinose containing polysaccharide moiety and a protein part.

They occur as minor components of flour (0.2 - 0.4 % w/w). Arabinogalactan-proteins are highly branched structures in which galactose residues are glycosidically bonded to each other with β-(1->3) - and β-(1->6)-linkages. Single arabinose units are bonded β-glycosidically to the galactan chain. Due to its high degree of branching the arabinogalactan-peptide is highly soluble in water, in aqueous ethanol or ammonium-sulphate solutions.

A proposed model of arabinogalactan-protein from wheat is presented in Figure 1.

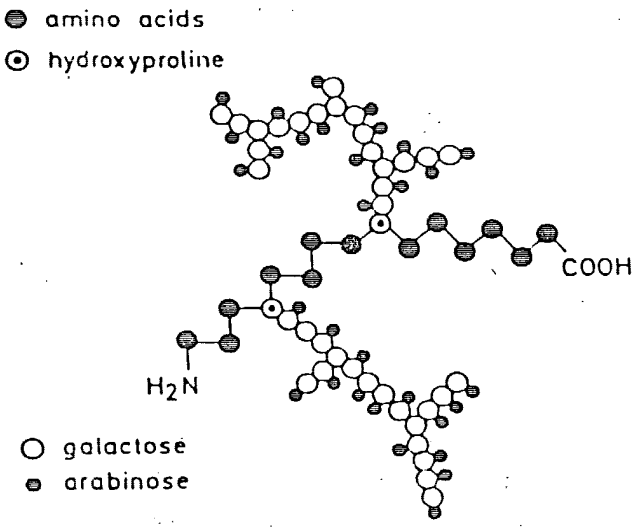

Figure 1. Possible structure for arabinogalactan-protein of wheat (Strahm et. al., 1979)

According to this proposal, the basic chain of galactose is branched. However, others have suggested a structure in which branching occurs at the peptide sites while the galactan chains themselves are unbranched.

1.2 Depolymerisation of arabinogalactan

Several enzymes are required to obtain a complete depolymerisation of arabinogalactan: arabinofuranosidase releasing arabinose from the galactan chain, galactanases hydrolysing β-(1->3)- and β-(1->6)-linked galactose residues and β–galactosidase liberating single galactose units.

Depolymerisation of arabinogalactan can be followed by several methods, three different procedures were applied in this study.

Gel permeation chromatography was used to detect the conversion of high molecular weight compound into low molecular weight products, whereas the release of sugars was measured by Gas Liquid chromatography. Formation of oxidizable substrate was followed by galactose oxidase. This was done indirectly by oxidation of ABTS (2.2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)), Figure 2.

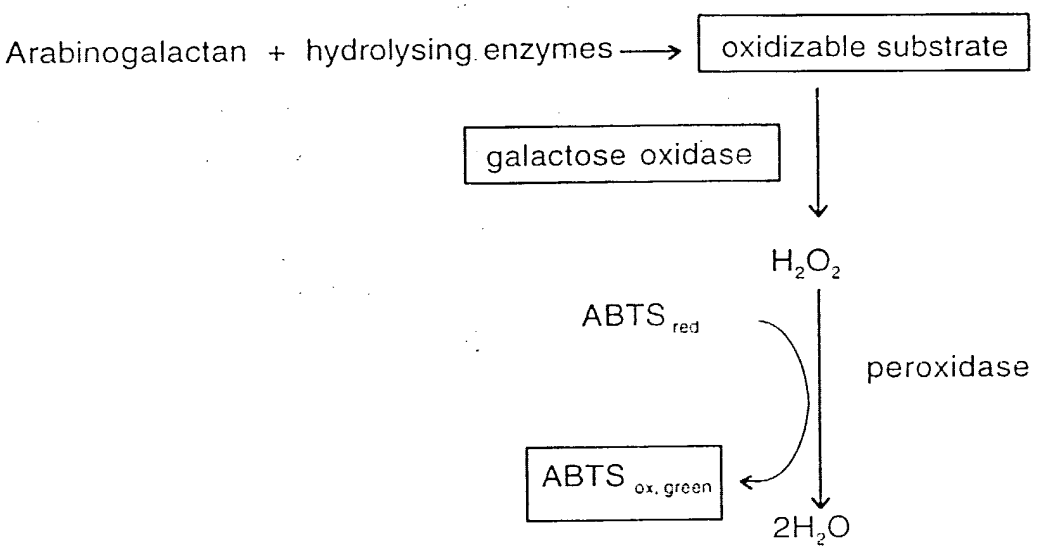

Figure 2. ABTS method.

Native arabinogalactan is not a substrate for galactose oxidase, but during depolymerisation substrates for galactose oxidase will be released. ABTS is being oxidized by produced hydrogen-peroxide from the enzyme reaction in the presence of a peroxidase. This results in a colored compound detectable spectrophotometrically at 420 nm. The absorbance is a function of the amount of available oxidizable substrate and the degree of favorisation of the substrate to associate with galactose oxidase.

2. Materials and methods

2.1 Isolation of arabinogalactan

6 g of water soluble pentosan from Thesee flour (Faurot et al., 1995) was dissolved in 150 ml Millipore water. The solution was adjusted with 96% ethanol to obtain a concentration of 60% (v/v) in order to precipitate arabinoxylan overnight.

The solution was centrifuged for 30 minutes at 4°C (12000 g) and the supernatant filtrated and adjusted to 80 % (v/v) ethanol. Arabinogalactan was precipitated and the solution was submitted to centrifugation for 30 minutes at 4°C (12000 g). The precipitate (arabinogalactan) was redissolved in Millipore-water and freeze dried giving a yield of 22 % on weight basis.

2.2 Enzyme treatment of arabinogalactan

Arabinogalactan (20 mg/ml) dissolved in 0.1 M Na-acetate buffer, pH 5.0 was incubated with different enzymes at room temperature.

The enzymes applied were: Pectolytic enzyme 2524-70 (p.70), Danisco Ingredients; Pectolytic enzyme 2524-71 (P.71), Danisco Ingredients; Grindamyl S 100 (Gr.S100), Danisco Ingredients; α-L-arabinofuranosidase (Megazyme) and arabinofuranosidase B (Jaap Visser).

Enzyme reactions were stopped by boiling for 5 minutes. The samples were centrifuged (12000 g) and the supernatant was used for further studies.

2.3 Oxidation of ABTS

ABTS reagent:

6.25 mg ABTS (A-1888,Sigma) dissolved in 10 ml 0.1 M Na-phosphate buffer, pH 6.4.

160 µl galactose oxidase (1 mg/ml), (G-7400, Sigma)

160 µl peroxidase (200 U/ml), (P-8125, Sigma)

0.1 M Na-phosphate buffer, pH 6.4. was added up to 25 ml.

800 µl ABTS reagent was incubated with 50 µl of enzyme-treated arabino-galactan in cuvettes for 30 minutes at room temperature.

The absorbance was immediately read at 420 nm.

2.4 Arabinofuranosidase assay

Fractionated p.70 was screened for arabinofuranosidase activity by the use p-Nitrophenyl α-L-arabinofuranoside (pNPA) from Sigma.

50 µl diluted enzyme solution were added to 0.5 ml of 1 mM pNPA dissolved in 50 mM Na-acetate buffer, pH 5.0.

Incubation took place for 30 minutes at room temperature. 1 ml of 1 M Na_2CO_3 was added and the yellow colour of released paranitrophenol could be detected at 410 nm in a spectrophotometer. Activities were calculated using a molar extinction coefficient of 13700 $M^{-1} * cm^{-1}$.

2.5 Preparation of galactan

Galactan was prepared by incubating arabinogalactan with high concentrations of arabinofuranosidase. 2 ml of arabinogalactan (20 mg/ml in 0.1 M Na-acetate buffer, pH 5.0) was incubated for 1 hour with 200 µl of arabinofuranosidase B (Jaap Visser) at room temperature. Galactan was precipitated in 85 % ethanol (v/v) for 2 hours. The solution was centrifuged at 1000 g for 15 minutes and the pellet was dried and redissolved in Millipore water.

2.6 Gel permeation chromatography (GPC)

Enzyme-treated arabinogalactan (500 µl arabinogalactan (20 mg/ml) + 10 µl p.70) was filtrated and applied for gel permeation chromatography, using a Waters system with refractometric detection.

100 µl was injected on a Superdex 75 HR 10/30 column (Pharmacia) at a flow rate of 1 ml/min in 0.1 M Na- acetate buffer, pH 5.0.

2.7 Gas liquid chromatography (GLC)

Preparations for GLC:

100 µl of enzyme-treated arabinogalactan, 100 µl internal standard (inositol, 1 mg/ml) and 900 µl Millipore water were mixed with 1 ml of 3 N H_2SO_4 .

Hydrolysis took place for 2 hours at 100 °C, the samples were cooled and 0.5 ml ammonia, 25% was added.

1 ml of 0.02 g/ml of $NaBH_4$ dissolved in DMSO was added to 150 µl of the hydrolysed samples.The solutions were incubated for 90 minutes at 40 °C before addition of 100 µl acetic acid (glacial) and cooling.

The reduced samples were acetylated by addition of 200 µl 1-methylimidazole and 2 ml of acetic acid anhydride for 20 minutes at room temperature. 6 ml of Millipore water was added and the solutions were well mixed.

The derivatized sugars were extracted by 2 ml of chloroform and washed 3 times with 8 ml of water. 1 ml of sample was transferred to an Eppendorf tube and let to evaporate over night. The samples were redissolved in 200 µl of chloroform and 4 µl was injected on a fused-silica DB-225 capillary column (30 x 0.32 µm i.d., 0.25 mm film; J & W Scientific) at 200°C with H_2 as the carrier gas.

3. Results

3.1 Screening for production of oxidizable substrates for galactose oxidase

Three different enzymes, p.70, p.71 and Gr.S100 were screened by the use of the ABTS assay for the production of oxidizable products when incubated with arabinogalactan.

250 µl of arabinogalactan (20 mg/ml) was incubated with 5 µl p.70, 5 µl p.71 or 5 µl Gr.S100 (10 mg/ml) for 90 min at room temperature, pH 5.0.

Only p.70 acting on arabinogalactan yielded oxidizable products for galactose oxidase. p.71 and Gr. S100 gave practically no response in the oxidation of ABTS. No synergistic effects were observed by the combination of the enzymes (Figure 3). It has to be mentioned however that p.71 yielded oxidizable substrates at an experiment performed at a lower pH (pH 3).

Further investigations of depolymerisation of arabinogalactan were performed with p.70.

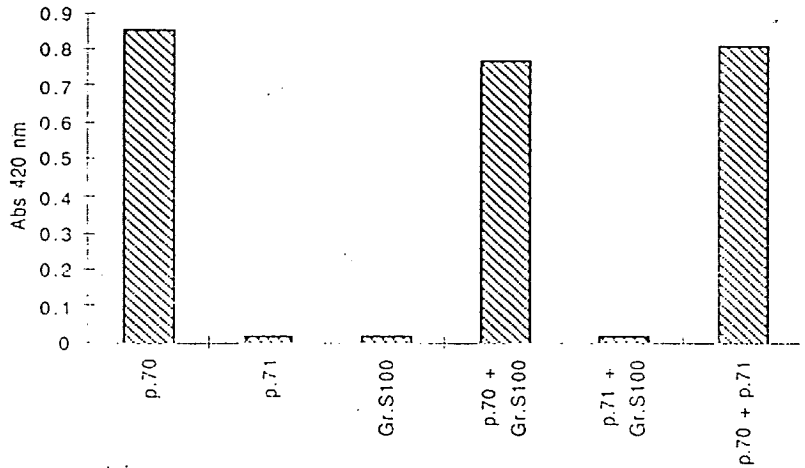

Figure 3. ABTS oxidation of enzyme-treated arabinogalactan by Pectolytic enzymes 2524-70 (p.70), Pectolytic enzymes 2524-71 (P.71) and Grindamyl S 100 (Gr.S100).

3.2 pH optimum for the production of oxidizable substrates by p.70

The pH optimum for the production of oxidizable substrates by p.70 was determined by incubating arabinogalactan with p.70 at different pH values ranging from pH 3 to pH 7, and resulting oxidizable products were determined by the ABTS assay.

The relative production of oxidizable substrates by p.70 on arabinogalactan versus pH is presented in Figure 4. Maximum actvity was observed around pH 5, and high levels of activity between pH 3.5 and 6. At pH 3 was 34 % activity detected and pH 7 resulted in only 12 % activity.

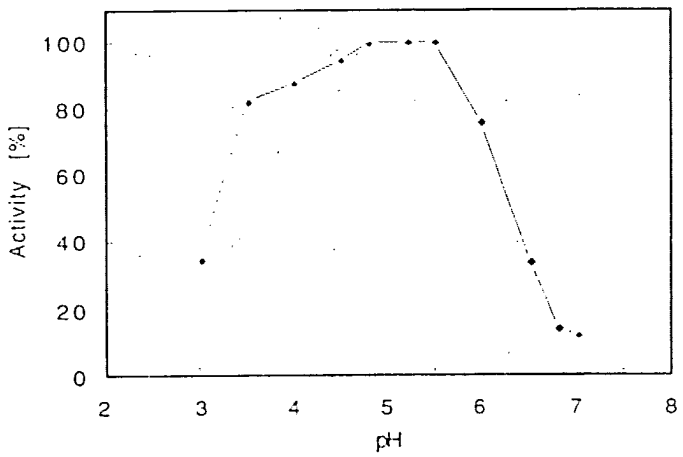

Figure 4. The relative production of oxidizable substrates by p.70 on arabinogalactan versus pH.

3.3 Release of oxidizable substrate during incubation of p.70 with arabinogalactan

Arabinogalactan (300 µl, 20 mg/ml) was incubated with p70. (5 µl) and the enzyme reaction was stopped at different time intervals in order to determine the production course of oxidizable substrate for galactose oxidase by the ABTS assay.

The results are presented in Figure 5. Production of oxidizable substrates increased fast from 0 to 15 minutes of reaction time. At 30 min of incubation time, maximum oxidation by galactose oxidase was observed, the oxidation herafter decreased slowly.

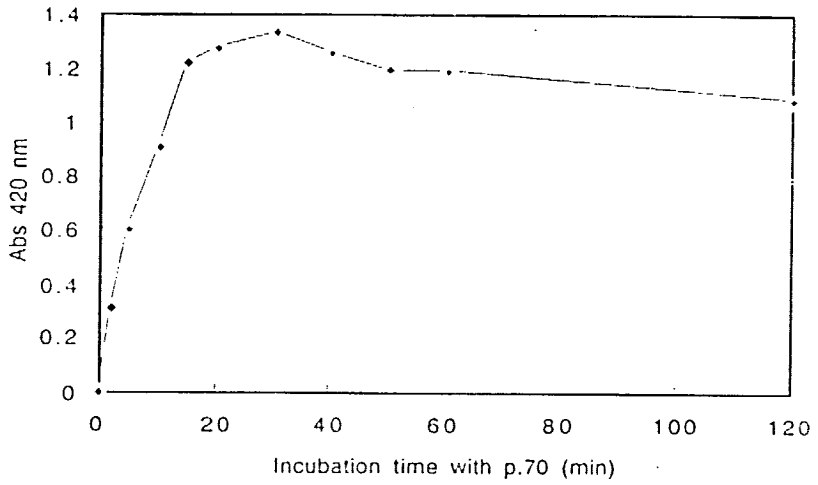

Figure 5. Oxidation of p.70 treated arabinogalactan by galactose oxidase at different incubation times, determined by ABTS assay.

3.4 Depolymerisation of arabinogalactan followed by GPC

In order to follow the depolymerisation of arabinogalactan by p.70, arabinogalactan exposed to p.70 with different incubation times was applied to gel permeation chromatography.

The chromatographic profiles of arabinogalactan incubated with p.70 for 30 minutes and 18 hours of reaction time and arabinogalactan exposed to inactivated p. 70 are compared in Figure 6.

High molecular weigt molecules were eluted in the non-degraded arabino-galactan sample. After 30 minutes of reaction time, a shift towards smaller molecules was seen and after 18 hours of reaction time with p. 70 almost all high molecular compounds were degraded into small molecules or simple sugars.

The GPC profiles were divided into five fractions representing different molecular size of depolymerized arabinogalactan. The enzyme-treated arabinogalactan was injected again and collected in fractions according to the five fractions.

The collected fractions were then submitted to gas chromatography and to ABTS oxidation.

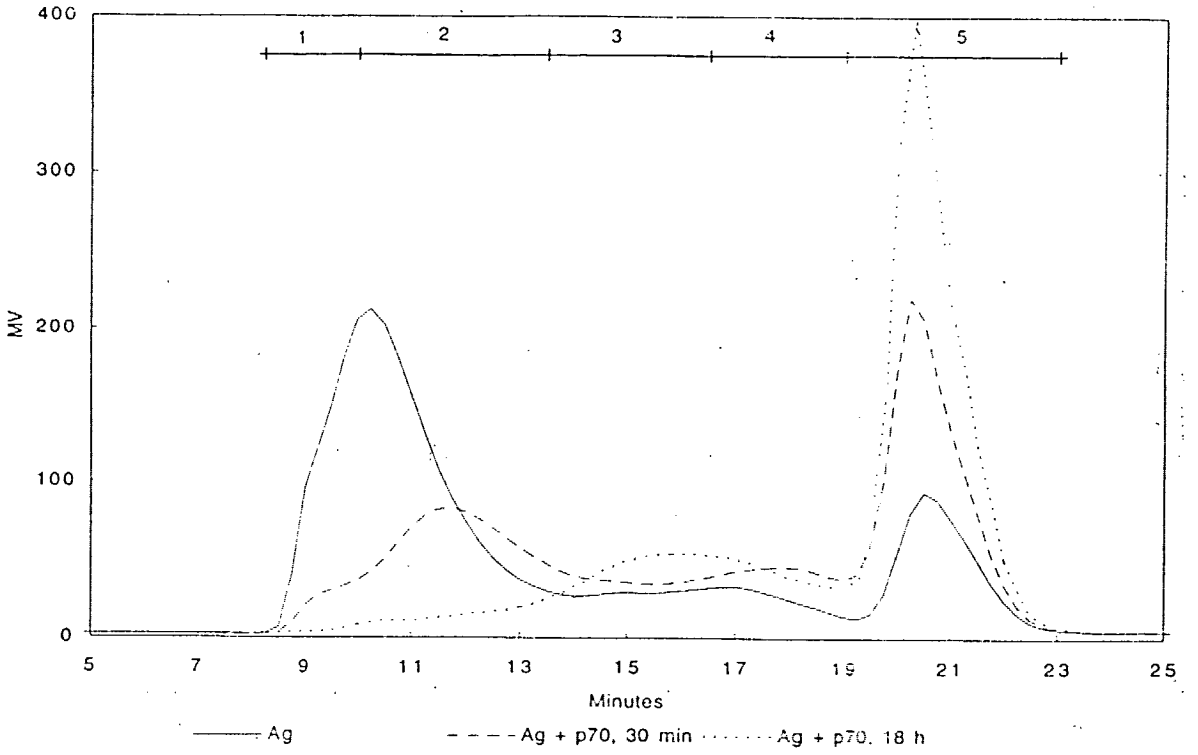

Figure 6. GPC profiles of arabinogalactan incubated with p.70 for 30 minutes and 18 hours of reaction time and arabinogalactan exposed to inactivated p.70.

3.4.1 Sugar distribution in collected fractions from GPC

The sugar distribution in the five collected fractions from GPC was determined by gas liquid chromatography with respect to arabinose and galactose. The fractions were submitted to hydrolysis to obtain simple sugars, derivatised and injected on the column.

The amount of galactose and arabinose from undegraded arabinogalactan, arabinogalactan incubated 30 minutes and 18 hours with p.70 are compared in Figure 7.

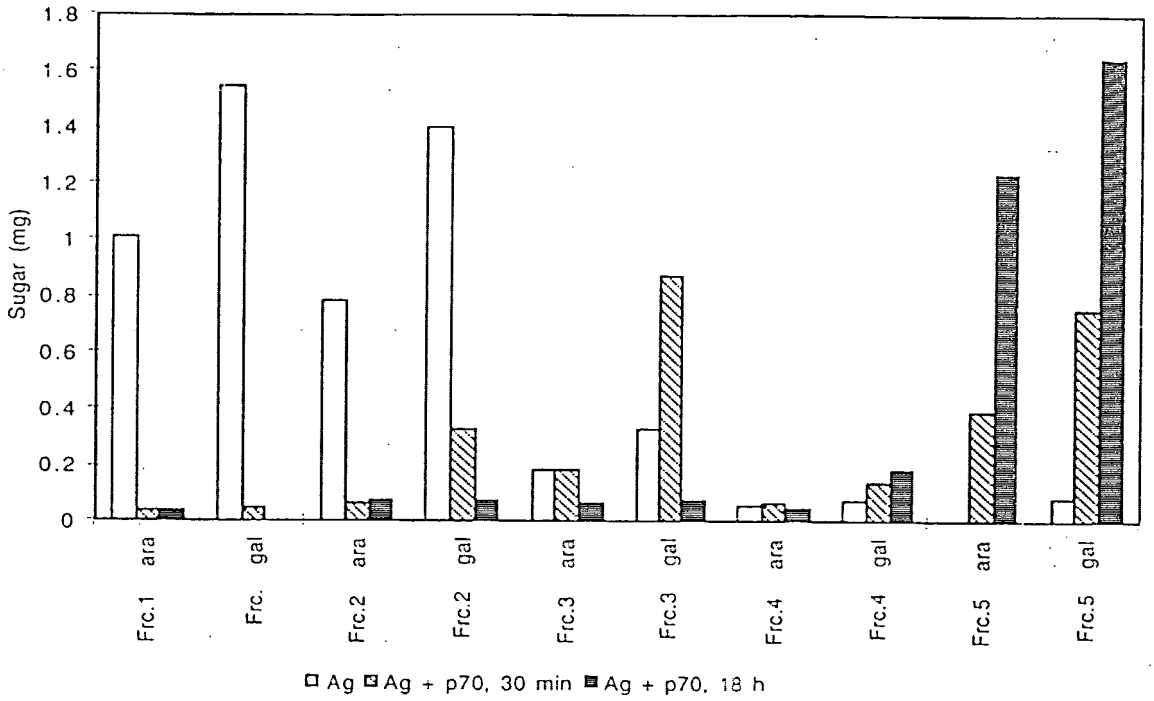

Figure 7. Sugar distribution in collected fractions from GPC from undegraded arabinogalactan, arabinogalactan incubated 30 minutes and 18 hours with p.70.

High amounts of galactose and arabinose were present in fraction 1 (representing high MW compounds) in undegraded arabinogalactan. This was not observed for degraded arabinogalactan after 30 min and 18 hours incubation with p.70.

On the other hand, high amounts of galactose and arabinose were present in fraction 5 (representing small molecules and free sugars) after 30 min and 18 hours of incubation with p.70.

When arabinogalactan had been incubated with p.70 for 30 minutes relatively high amounts of galactose were observed in fraction 2 and 3, representing molecules of intermediate size.

3.4.2 ABTS oxidation of collected fractions from GPC

The five collected fractions from GPC were analysed by the ABTS assay. This was done in order to determine their ability as substrate for galactose oxidase. The degradation products from arabinogalactan representing fraction 2 and 3 from 30 minutes of degradation by p.70 were - very surprisingly - the only

fractions that were oxidizable. Released galactose from the depolymerisation should be present in fraction 5 (after 30 minutes and 18 hours of incubation), but this fraction gave no response in oxidation of ABTS.

The absorbances at 420 nm in the ABTS assay from the different fractions are shown in Figure 8.

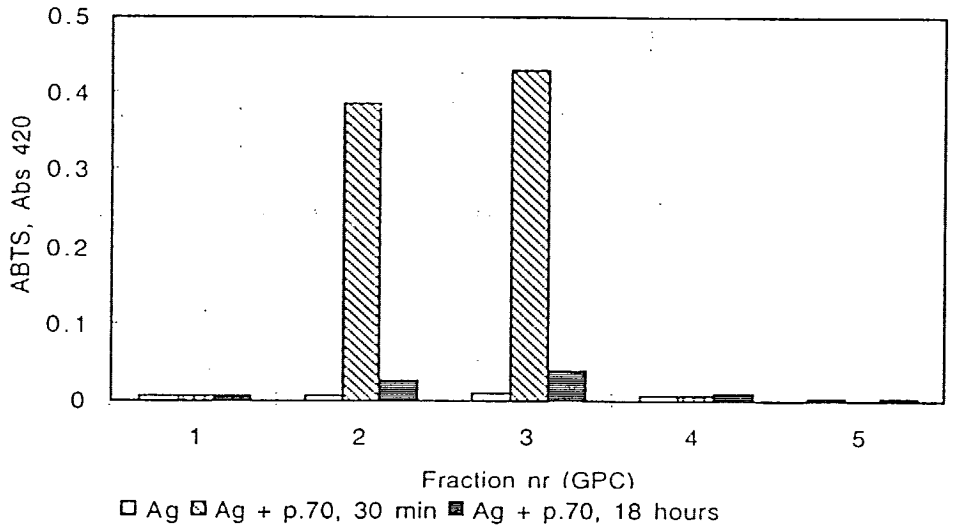

Figure 8. Yield in absorbance at 420 nm in the ABTS assay by the collected fractions from GPC (undegraded arabinogalactan, arabinogalactan incubated 30 minutes and 18 hours with p.70)

3.5 Production of galactose and oxidizable substrate at different concentrations of p.70 acting on arabinogalactan

300 µl arabinogalactan (20 mg/ml) was exposed to 50 µl of different dilutions of p.70 for 45 min at room temperature. The resulting oxidizable products were determined by the ABTS assay and the release of galactose and arabinose were determined by GLC.

Increasing dilution of p.70 resulted in an increase of oxidizable substrate up to 30 times of dilution (Figure 9), this was not expected and did not correspond to the production of galactose measured by GLC. The release of galactose and arabinose decreased with increasing dilutions of p.70 (Figure 10). The undiluted p.70 released 80% of the total amount of galactose present in the applied arabinogalactan.

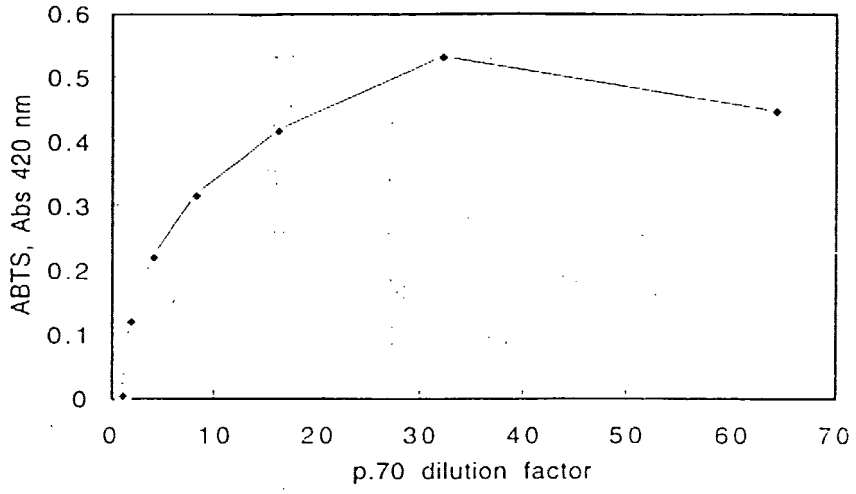

Figure 9. Yield in absorbances at 420 nm in the ABTS assay of arabinogalactan incubated with different dilutions of p.70.

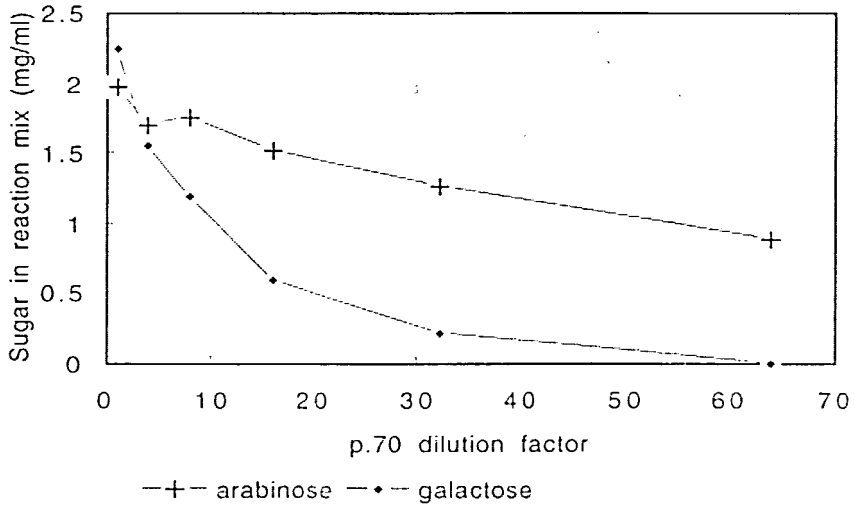

Figure 10. Release of galactose and arabinose as a function of different dilutions of p.70 on arabinogalactan.

3.6 Inhibition of galactose oxidase activity

The observed decrease in oxidation by galactose oxidase by increasing concentrations of p.70 (Figure 9) lead to the suspicion of the presence of an inhibitor in the p.70 enzyme solution.

Degraded arabinogalactan was therefore incubated with pronase in order to eliminate an eventual oxidase inhibitor of protein nature before measuring the amount of oxidizable substrate by ABTS. No difference were detected between pronase and not-pronase treated arabinogalactan.

While Cu^{2+} is essential for galactose oxidase activity, ABTS oxidations were performed in 0.5 mM $CuSO_4$ in order to resist an eventual chelating of cupper by agents present in the enzyme solution. No differences were observed between ABTS experiments performed with and without $CuSO_4$.

In order to eliminate eventually small molecule inhibitors the p.70 was precipitated in ethanol and redissolved in buffer before incubation with arabinogalactan. This removal of small molecules resulted in an increase of oxidation by galactose oxidase.

3.7 Partial purification of p.70

With the purpose to isolate a galactanase, p.70 was fractionated by anion exchange chromatography on a Q-Sepharose fast flow column. This was done by Danisco Ingredients in Aarhus .

The different fractions were incubated with arabinogalactan and the release of oxidizable substrates by galactose oxidase was measured by the ABTS assay. The elution profile and the response in activity is presented in Figure 11. The ability to release oxidizable substrates were detected in a single peak, fractions 15-20.

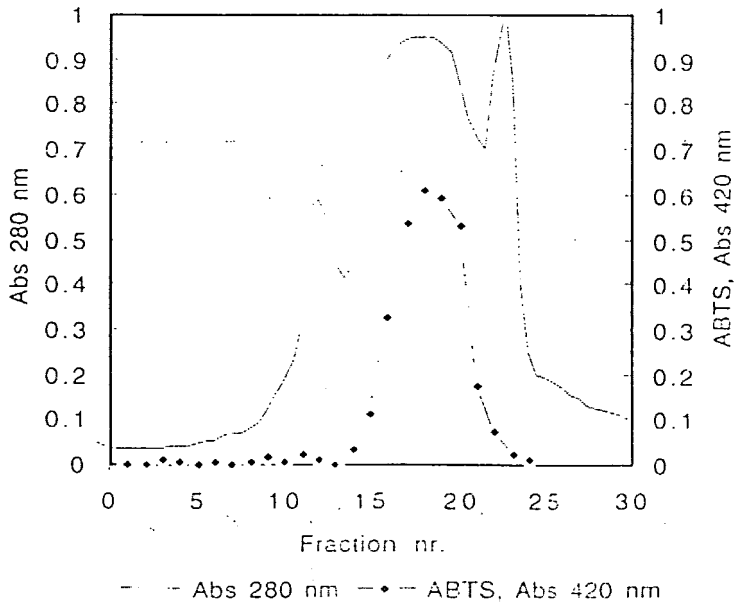

Figure 11. Elution profile of p.70 on a Q-Sepharose fast flow column.

The enzymatically active fractions were pooled and applied for further fractionating by hydrophobic interaction chromatography (HIC), also done by Danisco Ingredients in Aarhus. The elution profile and the response in the ABTS assay is presented in figure 12. Enzymatic activities releasing oxidizable substrates from arabinogalactan were detected in the run-through, a peak representing fractions 5-11 and in fraction 14 and 16.

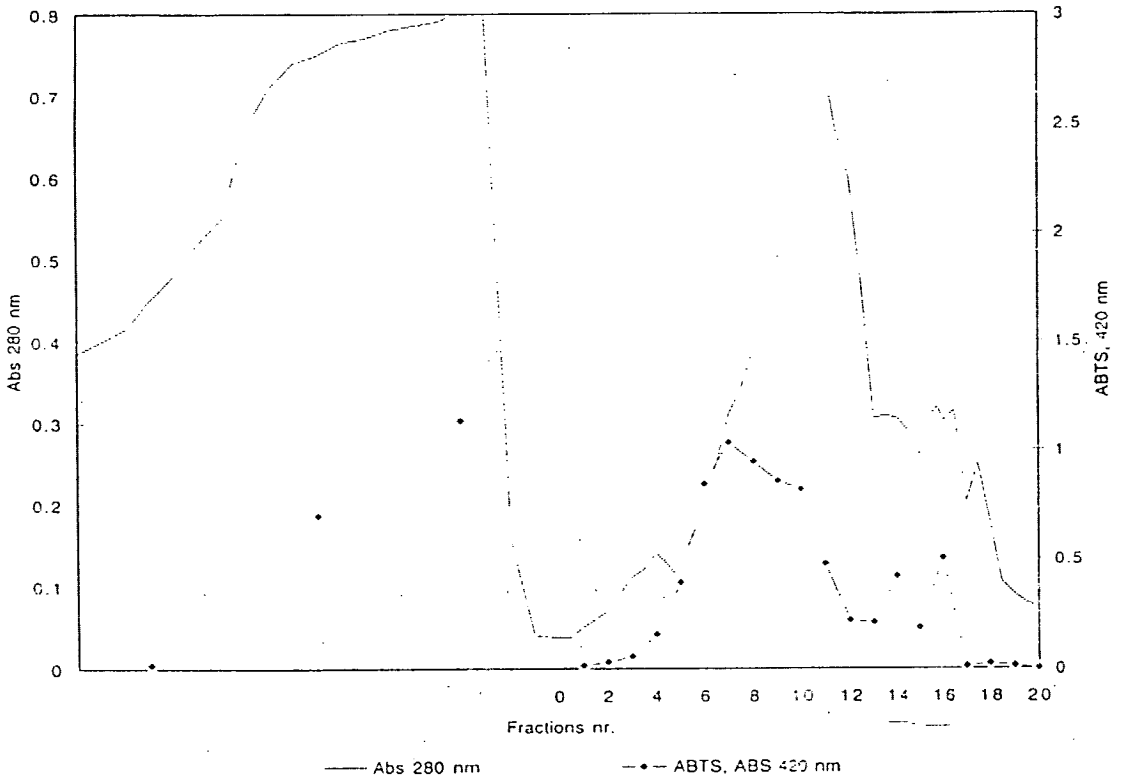

Figure 12. Elution profile of p.70 seperated by HIC.

The same fractions were also screened for arabinofuranosidase activities by the use of pNPA as subtrate. There was - very sursprisingly - a complete concordance between detected arabinofuranosidase activity and activities releasing oxidizable substrates for galactose oxidase in the different fractions, Figure 13.

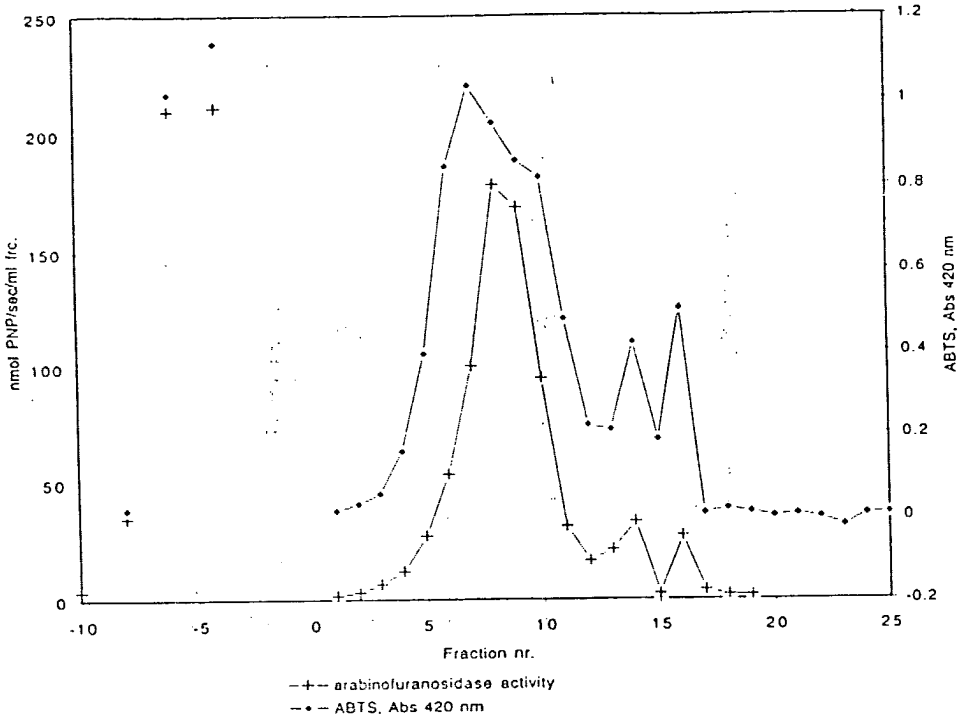

Figure 13. Comparison of detected arabinofuranosidase activity and activities releasing oxidizable substrates for galactose oxidase in fractionated p.70.

3.8 Oxidation of galactan by galactose oxidase

Galactan was applied to oxidation by galactose oxidase in the ABTS assay and compared to galactose as substrate. The corresponding concentration of galactose in the galactan solution was 4 mg/ml, the results showed that at the same level of galactose, free galactose yielded only 40 % of oxidation compared to galactan.

Three different concentrations of galactose were compared to galactan with respect to substrate capacity for galactose oxidase , Figure 14.

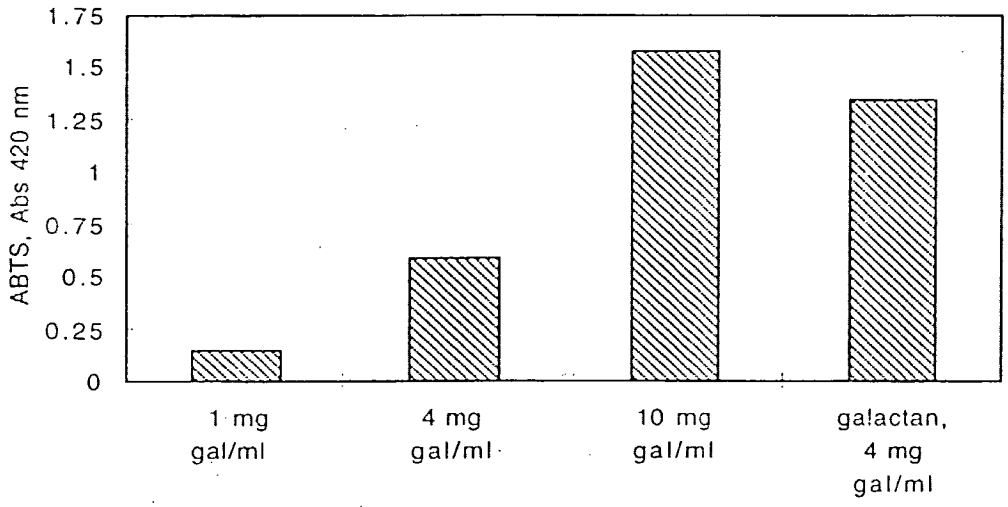

Figure 14. Galactose and galactan oxidized by galactose oxidase in the ABTS assay.

3.9 Release of oxidizable substrate and arabinose during incubation of arabinofuranosidase with arabinogalactan

Arabinogalactan was incubated with arabinofuranosidase (300 µl arabinogalactan (20 mg/ml) + 40 µl arabinofuranosidase, Jaap Visser) and the release of arabinose was followed by GLC, whereas the release of oxidizable substrate was followed by the ABTS assay. The results are presented in Figure 15.

The production of oxidizable substrate followed the release of arabinose, 80 % of the total amount of arabinose present in arabinogalactan was released after 90 minutes. It was confirmed that arabinose was not a substrate for galactose oxidase by the ABTS assay.

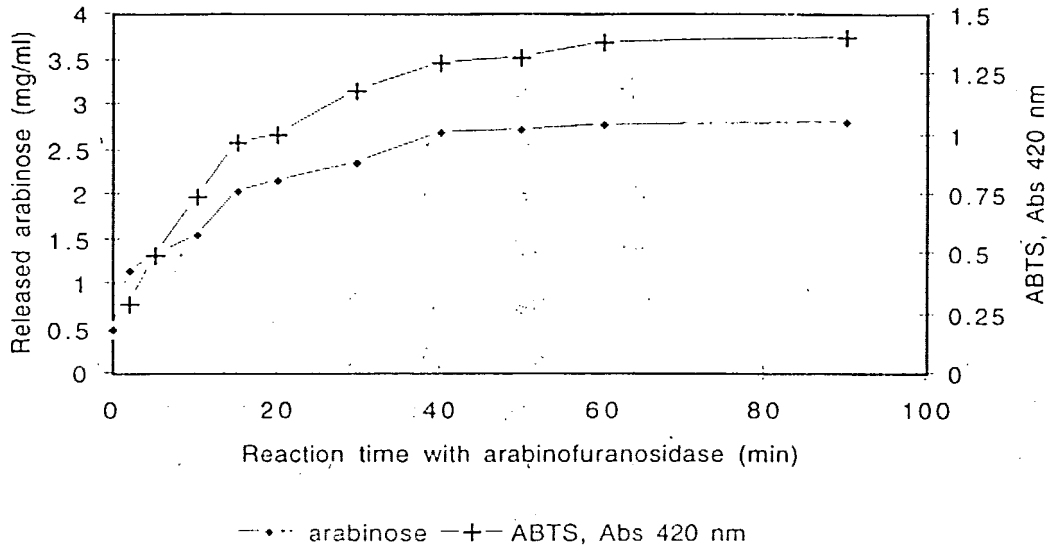

Figure 15. The release of arabinose compared to the oxidizability by galactose oxidase in the ABTS assay during incubation of arabinogalactan with arabinofuranosidase.

4. Discussion

The degradation of arabinogalactan with the purpose to liberate substrate for galactose oxidase have been investigated by the use of several enzymes.
Pectolytic enzyme 2524-70 (p.70), showed activities in the liberation of oxidizable substrate to galactose oxidase. This enzyme solution had a pH optimum around pH 5 and released increasing amounts of galactose with increasing concentrations of enzyme.
Gel permation chromatography showed that arabinogalactan was depolymerized into small molecules by the action of p.70, indicating the presence of arabinofuranosidase- and galactanase-activities in the enzyme solution.

The oxidation by galactose oxidase of the degradation products from arabinogalactan was followed by the ABTS assay and showed that increasing levels of p.70 lead to decreasing oxidation of ABTS. This was probably caused by an inhibitor present in the p.70 inhibiting either oxidation of substrate released by p.70 or the oxidation of ABTS by peroxidase.

This inhibitor was not of protein nature, neither an agent chelating cupper, but a small molecule precipitated by ethanol.

The course of liberation of oxidizable substrates during incubation with p.70 showed that maximum oxidation appeared in the beginning of incubation. Oxidation hereafter decreased with incubation time indicating that complete depolymerisation of arabinogalactan into galactose is not the most favorable for oxidation by galactose oxidase. This was supported by the oxidation of degradation products collected from GPC according to molecular weight. Fractions containing poly- and oligosaccharides were oxidized significantly better than small molecules including galactose. It has to be mentioned however that the previously mentioned inhibitor probably was eluted in the same fraction as galactose.

The concordance between detected arabinofuranosidase activity and activities releasing oxidizable substrates for galactose oxidase in fractionated p.70 lead to further investigations on the of effect of pure arabinofuranosidase on arabinogalactan. It was found that α-L-arabinofuranosidase B from *A. niger* was able to produce oxidizable substrate from arabinogalactan. The efficency as substrate increased by increasing release of arabinose.
Arabinose-released arabinogalactan was, in fact. twice as efficient as substrate compared to galactose. These results indicates that arabinogalactan does not have to be depolymerised in order to be used as substrate for galactose oxidase.

Galactose oxidase is, according to the litterature, able to act on some oligosaccharides with galactose in a terminal non-reducing position substantially faster than on galactose (Bretting et. al., 1986). Their investigations on the structural requirements for oligosaccharides to bind to galactose oxidase indicated that the enzymic active centre of galactose oxidase recognizes a determinant on galactan comprising more than one galactose residue.
It was found that galactose residues linked β-(1->6) were oxidized substantially faster than the corresponding β-(1->3) linked oligosaccharide and that the β-(1->3) or β-(1 ->6) linked determinant has to be in terminal position to be accessible to galactose oxidase.

A branched tetrasaccharide exposing two terminal galactose residues in a β-(1->3) and β-(1->6) linkage was the most complementary structure in the native galactan to associate with galactose oxidase. This compound was oxidized 10 times faster than galactose.

According to the question whether the galactan chain in arabinogalactan from wheat flour is branched or not, is the branching theory supported by these results. A branched chain will contribute to structural requirement for galactose oxidase activity through small oligomeric side chains.

Further investigations needs to be done in order to see if a purified galactanase from p.70 will improve the structural requirements for galactose oxidase by degrading the galactan chain into oligomers.

The ABTS assay using depolymerized arabinogalactan as substrate can not be used to distinguish galactanase activity from arabinofuranosidase activity, future assays have to be performed with alternatively methods.

Another interesting aspects would be to study a combination of arabino-furanosidase and β-galactosidase on arabinogalactan and see how this will contribute to oxidation by galactose oxidase.

Preliminary baking experiments have shown promising results, both in dough characteristics and bread volume. A baking experiment, where a combination of p.70 and galactose oxidase was applied, resulted in 50 % bread volume increase and a fine dough quality. Results obtained with galactose oxidase were all better than results obtained with glucose oxidase.

5. References

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